U. S. DEPARTMENT OF AGRICULTURE.

BUREAU OF PLANT INDUSTRY—BULLETIN NO. 211.

B. T. GALLOWAY, *Chief of Bureau.*

BACTERIOLOGICAL STUDIES OF THE SOILS OF THE TRUCKEE-CARSON IRRIGATION PROJECT.

BY

KARL F. KELLERMAN,

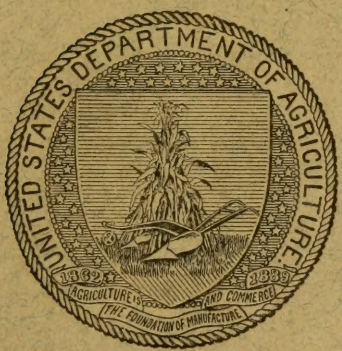
Physiologist in Charge of Soil-Bacteriology and Water-Purification Investigations,

AND

E. R. ALLEN,

Scientific Assistant.

ISSUED APRIL 15, 1911.



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BUREAU OF PLANT INDUSTRY.

Chief of Bureau, BEVERLY T. GALLOWAY.
Assistant Chief of Bureau, WILLIAM A. TAYLOR.
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SOIL-BACTERIOLOGY AND WATER-PURIFICATION INVESTIGATIONS.

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Plant Industry,
Office of the Chief,
Washington, D. C., January 17, 1911.

Sir: I have the honor to transmit herewith a paper entitled "Bacteriological Studies of the Soils of the Truckee-Carson Irrigation Project" and to recommend that it be published as Bulletin No. 211 of the series of this Bureau.

These investigations, though in many ways of a preliminary character, indicate some of the possibilities of a bacteriological diagnosis of soils and will be of interest to all who have to deal with problems of soil fertility.

Respectfully,

Wm. A. Taylor,
Acting Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

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BACTERIOLOGICAL STUDIES OF THE SOILS OF THE TRUCKEE-CARSON IRRIGATION PROJECT.

INTRODUCTION.

In making a bacteriological study of any soil or group of soils there are certain fairly well defined groups of micro-organisms whose functions, although as yet imperfectly understood, are recognized as important factors in crop production and are more or less familiar to everyone who has attempted to investigate the problems of soil fertility. These groups of micro-organisms may be roughly separated into four classes, depending upon their physiologic characteristics: (1) Parasites, or organisms important chiefly because they are pathogenic to animals or plants and are frequently found in soils; (2) the cellulose-destroying organisms; (3) the organisms associated with the formation of humus; and (4) the organisms associated with the transformation of soil nitrogen. Only those groups concerned with the transformation of nitrogen, which in the form of ammonia or nitrate is practically the most important of all plant foods, are reported upon at this time.

The data sought in studies of this character may be outlined as follows:

(1) Total numbers of saprophytic bacteria in measured quantities of soil.
(2) Ammonification; the breaking down of nitrogenous organic matter into ammonia.
(3) Nitrification; the oxidation of various compounds of nitrogen to nitrate.
(4) Denitrification; the reverse of nitrification.
(5) Nitrogen fixation, symbiotic and nonsymbiotic; the utilization of atmospheric nitrogen in forming nitrogenous organic compounds.

In the work conducted at Fallon, Nev., during the season of 1909, in cooperation with the Office of Western Agricultural Extension, no quantitative study was made of nitrogen fixation, and the data on the subject of ammonification are very meager. Some preliminary investigations in arid regions had shown that nitrification takes place here at considerable depth. All studies, therefore, were made of a 3-foot zone, keeping separate the samples of soils from different depths.

The comparative nitrifying power of the different samples from the various plats is shown by curves, the parts per million of nitrogen as nitrate and nitrite being plotted as ordinates, and the different depths as abscissæ. These curves show only the gain in nitric and nitrous nitrogen. Chlorids and sulphates are also shown, but seem to be of

little importance. The quantity of nitric nitrogen originally present is shown in the legends under the diagrams (figs. 2–13).

A description of the Truckee-Carson Experiment Farm, at Fallon, Nev., upon which practically all of the work herein reported was conducted, is given in a previous bulletin of this Bureau.[1] The designations of the small plats from which samples were taken for bacteriological study and their location are shown in figure 1.

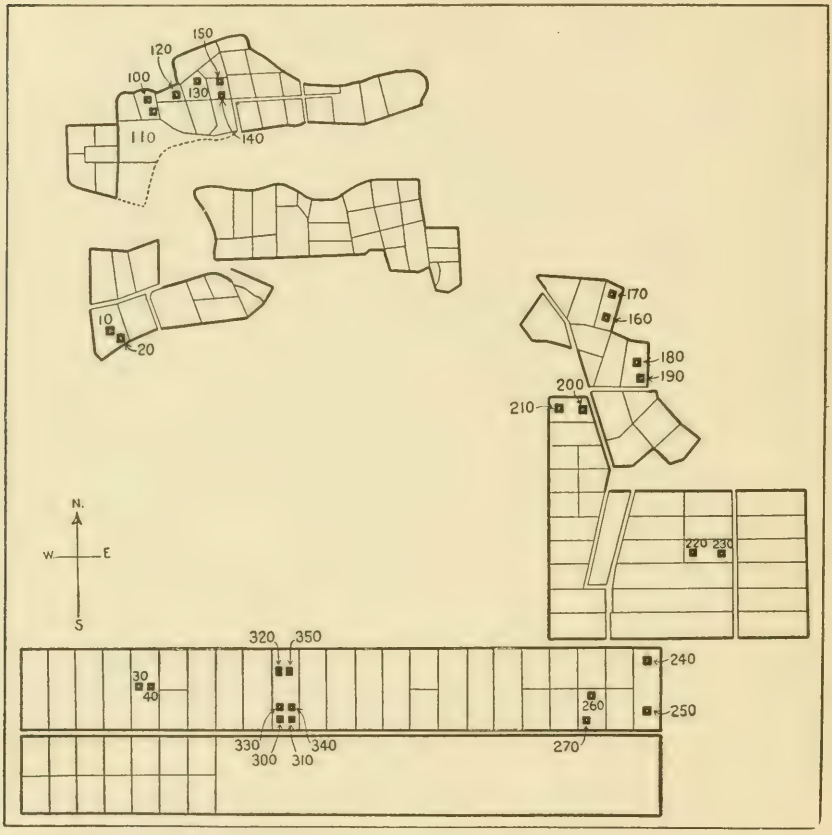

FIG. 1.—Location of sampling plats in the experimental fields of the Truckee-Carson Experiment Farm south of Fallon, Nev.

METHODS EMPLOYED IN BACTERIOLOGICAL INVESTIGATIONS OF THE SOIL AT FALLON, NEV.

REQUIREMENTS TO BE MET.

Investigations in soil bacteriology require first of all the selection and development of satisfactory methods for determining the distribution and activity of the micro-organisms which may occur under

[1] Scofield, C. S., and Rogers, S. J. The Truckee-Carson Experiment Farm. Bulletin 157, Bureau of Plant Industry, 1909.

different soil conditions. Though it is recognized that the methods suggested by different investigators are not adequate for accurate quantitative investigations of bacterial functions and conditions in various soils, the methods which at this time have been found most convenient and suitable for the investigations under discussion are briefly reviewed.[1]

COUNTS OF BACTERIA.

Samples of soil were collected with as strict aseptic precautions as it is possible to observe under field conditions. Sterile salt-mouth bottles were used as containers, and the soil auger used for taking up the soil was carefully cleaned and flamed over an alcohol lamp before sampling each stratum. In the laboratory 1-gram portions were removed from the bottles with a sterile scoop which held the required quantity, transferred to 300 cubic centimeters of sterile water in 500-cubic-centimeter flasks, and the whole shaken thoroughly at short intervals for fifteen minutes. One-cubic-centimeter portions of these infusions were then removed with sterile pipettes and added to 10 cubic centimeters of melted beef agar, and plates poured in the ordinary manner and incubated at 28° C. Counts of bacteria were made at the end of five-day periods.

AMMONIFICATION.

Sterile peptone solutions having the following composition were inoculated with 5 per cent of soil and the ammonia determined at the end of seven and fifteen days by distillation with magnesia:

Peptone	15 grams.
Dipotassium phosphate	3 grams.
Magnesium sulphate	3 grams.
Sodium chlorid	3 grams.
Water	1,000 c. c.

[1] Lipman, J. G. Experiments on the Transformation and Fixation of Nitrogen by Bacteria. Twenty-fourth Annual Report, New Jersey State Agricultural Experiment Stations, 1903, pp. 217–285.

Lipman, J. G., and Brown, Percy E. Methods Concerning Ammonia Formation in Soils and Culture Solutions. Report, Soil Chemist and Bacteriologist, New Jersey Agricultural College Experiment Station, 1908, pp. 95–105.

Lipman, J. G., and Brown, Percy E. Notes on Methods and Culture Media. Report, Soil Chemist and Bacteriologist, New Jersey Agricultural College Experiment Station, 1908, pp. 129–136.

Lipman, J. G. Azotobacter Studies. Report, Soil Chemist and Bacteriologist, New Jersey Agricultural College Experiment Station, 1908, pp. 137–143.

Löhnis, F. Ein Beitrag zur Methodik der bakteriologischen Bodenuntersuchung. Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten, pt. 2, vol. 12, no. 6–8, pp. 262–267, June 24, 1904; no. 11–16, pp. 448–463, July 14, 1904; vol. 17, no. 14–16, pp. 518–528, December 7, 1906; vol. 20, no. 24–25, pp. 781–799, April 15, 1908; vol. 24, no. 5–7, pp. 183–192, August, 1909.

Remy, Theodor. Bodenchemische und Bakteriologische Studien. Landwirtschaftliche Jahrbücher, vol. 35, Supplement 4, pp. 1–62. Berlin, 1906.

NITRIFICATION.

Samples of soil were collected with the precautions previously described. In some cases 1-gram portions for counts of total numbers of bacteria were removed from the bottle of soil and the remainder of the sample used for nitrification studies.

Because of the great variation in the fertilty of different fields it was considered necessary to determine at what depths the nitrifying bacteria existed; therefore, instead of emptying the soil from the container and allowing it to dry, thus exposing it to some contamination, one-half of the soil, approximately 50 grams, was removed with a sterile spatula and used for "original" determinations. Five cubic centimeters of 0.4 per cent ammonium sulphate was then added to the portion remaining in the bottle and the sample placed in the incubator at 28°C. With the original moisture of the soil this additional 5 cubic centimeters frequently made the water content of the soil somewhat above optimum, but owing to the rapid evaporation in an arid climate this rapidly decreased and was adjusted as nearly as possible in subsequent waterings. All samples were weighed at 3-day intervals, and as any appeared to fall below optimum the required quantity of sterile distilled water was added to restore them. The incubation period was two weeks, the temperature being maintained at 28°C.

The chemical work presented no little difficulty. The analytical determinations may be considered in two phases: (1) The preparation of the aqueous extract of the soil both before and after incubation with ammonium sulphate and (2) the determination of nitrites and nitrates in original and incubated samples.

In the preparation of the aqueous extract considerable difficulty was experienced. All of the soils used contained variable and frequently quite large proportions of very fine clay, which would not settle out and leave a clear supernatant liquid, even on prolonged standing. It was thought advisable to determine the chlorids and sulphates in the original samples; therefore the common salts containing these radicals could not be used to flocculate the clay, although this method was sometimes used in the examination of the samples after incubation where only nitrites and nitrates were determined. Pressure-pump facilities were inadequate for the large number of samples used, the more so as the fine clay particles clogged the porcelain filter and caused filtration to be extremely slow with the low pressure available.[1] Heating the sample in the oven at different temperatures previous to adding the water seemed to have no effect, so the supernatant liquid was first drawn off turbid, evaporated to dryness, baked at 90° to 100° C., and then filtered. In all of the

[1] Approximately 25 pounds to the square inch.

baking experiments it was noticed that the nearer a set of samples was baked at 100° C. the better the subsequent filtering, probably indicating that the clay is siliceous.

The Griess method is the standard for determining nitrites, but owing to the delay in getting chemicals at Fallon the potassium-iodid-starch method was used for a large part of the work. This method, while primarily a qualitative one, was found to be fairly reliable for quantitative determinations if a large quantity of reagent was used when the nitrites were high, as indicated by a rapid development of the blue-black color. The Grandval-Lajoux phenol-sulphonic acid method as modified by Syme [1] was used for estimating nitrates; before determining nitrates the nitrites were removed by urea in acid solution in accordance with Piccini's method.

Chlorids were frequently high in soil solutions in which nitrates were to be determined, and it was necessary to remove them when present in concentrations greater than 50 or 70 parts per million. This was accomplished by the use of silver sulphate.

Chlorids [2] were determined by the Mohr method, titrating the neutral solution with N/10 silver nitrate and using potassium chromate as an indicator. Sulphates [2] were determined by the turbidity method described by the Bureau of Soils.[3]

DENITRIFICATION.

Studies of denitrification were made by inoculating Dunham's peptone solution containing 0.2 per cent potassium nitrate with soil and with a Frost scale measuring roughly the quantity of free nitrogen evolved. Either ordinary fermentation tubes or test tubes inverted in salt-mouth bottles were used. The latter method is preferred, as it permits the use of larger quantities of soil for inoculations.

NITROGEN FIXATION.

Leguminous plants were examined for the presence of nodules, and Azotobacter cultures were isolated from soil samples.

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[1] Syme, W. A. The Colorimetric Determination of Nitrates in Soil Solutions Containing Organic Matter. Thirty-first Annual Report of the North Carolina Agricultural Experiment Station, for the Year Ending June 30, 1908, pp. 64–65.

[2] Both of these salts were determined by Mr. C. A. Jensen, of the Office of Western Agricultural Extension of the Bureau of Plant Industry.

[3] Schreiner, Oswald, and Failyer, George H. Colorimetric, Turbidity, and Titration Methods Used in Soil Investigations. Bulletin 31, Bureau of Soils, U. S. Dept of Agriculture, 1906.

NITRIFYING POWER OF SOILS AT DIFFERENT DEPTHS.

In investigations in soil bacteriology in the eastern United States only the surface soil shows great variations. The soil of the arid sections is much deeper, however; that is, the subsoil is less "raw" than in regions of heavier rainfall, a fact that has come to be more or less familiar to everyone studying soil conditions over extensive areas.

Figure 2 shows the nitrification of samples from plats 100 and 110. These plats, which are practically duplicates, are in a productive

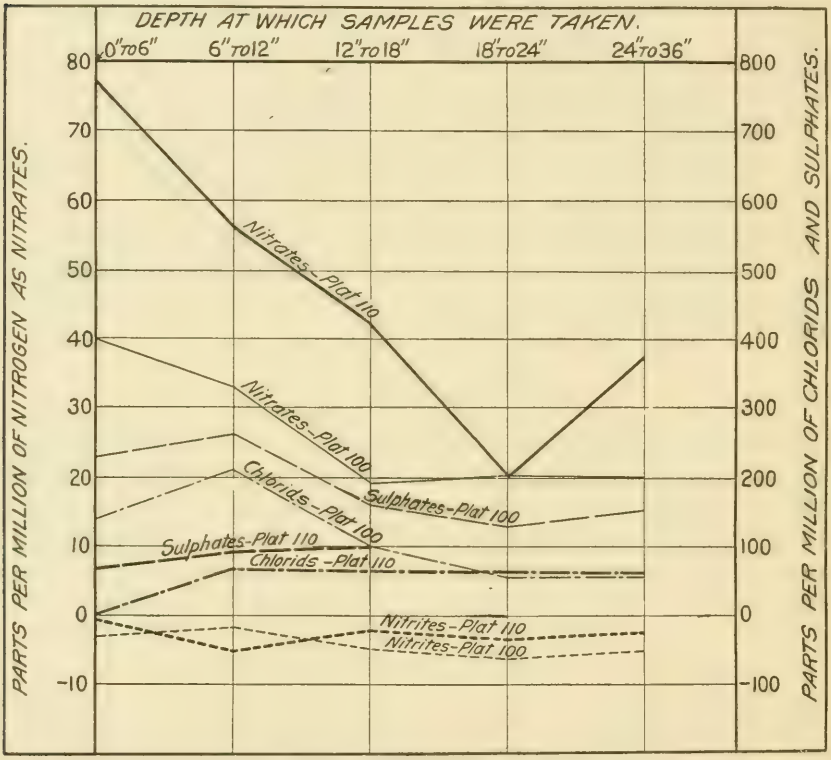

FIG. 2.—Diagram showing the nitrification of ammonium sulphate in samples of soil from different depths from plats 100 and 110, Truckee-Carson Experiment Farm. Original nitrate present in samples from plat 100: Depth, 0 to 6 inches, 8 parts per million; 6 to 12 inches, 15; 12 to 18 inches, 9; 18 to 24 inches, 4.8; 24 to 36 inches, 6.56. From plat 110: Depth, 0 to 6 inches, 9 parts per million; 6 to 12 inches, 7.4; 12 to 18 inches, 5.2; 18 to 24 inches, 4.8; 24 to 36 inches, 3.12.

alfalfa field which has been under cultivation for several years. The soil is loose and sandy throughout the 3-foot depth. The nitrate curves show that there is a gradual decrease in nitrifying power with depth.

Figures 3 and 4 show the nitrification in samples from plats 120 and 130. These are in a fertile alfalfa field similar to the one mentioned

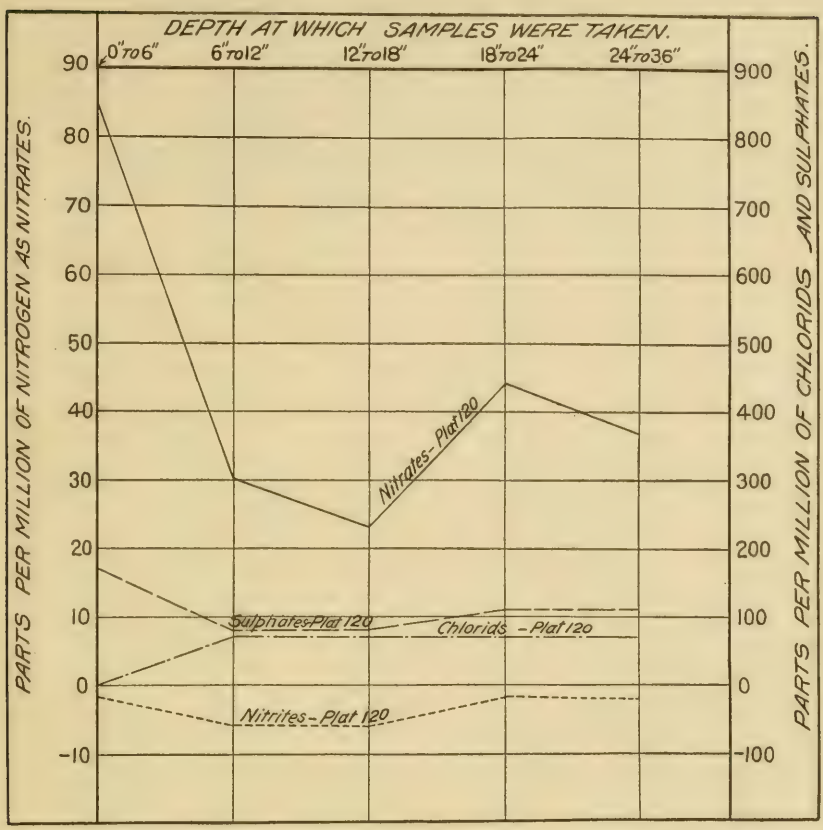

FIG. 3.—Diagram showing the nitrification of ammonium sulphate in samples of soil from different depths from plat 120, Truckee-Carson Experiment Farm. Original nitrate present in samples: Depth, 0 to 6 inches, 15.36 parts per million; 6 to 12 inches, 8.64; 12 to 18 inches, 6.72; 18 to 24 inches, 3.84; 24 to 36 inches, 2.88.

in the previous paragraph. The samples from plat 120 show nitrification varying rather irregularly with depth. Samples from plat 130

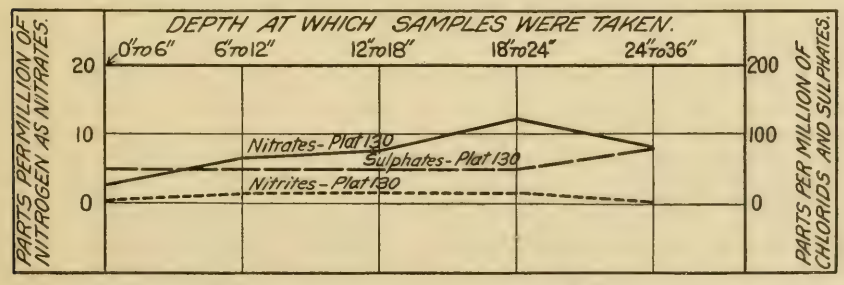

FIG. 4.—Diagram showing the nitrification of ammonium sulphate in samples of soil from different depths from plat 130, Truckee-Carson Experiment Farm. Original nitrate present in samples: Depth, 0 to 6 inches, 13.3 parts per million; 6 to 12 inches, 6.72; 12 to 18 inches, 9.6; 18 to 24 inches, 7.23; 24 to 36 inches, 14.4.

practically failed to nitrify,[1] although the two plats appear to be very similar.

Figure 5 shows the relative nitrifying power of good and poor soils collected from adjoining plats. Plat 160 has a loose sandy soil to a

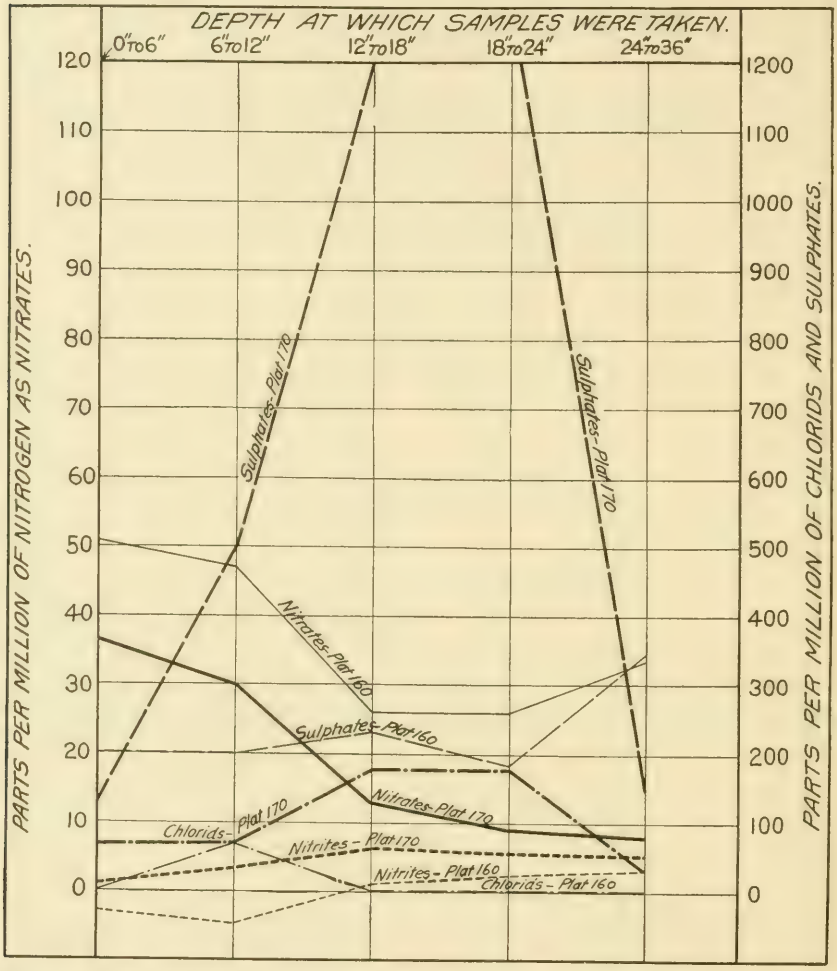

FIG. 5.—Diagram showing the nitrification of ammonium sulphate in samples of soil from different depths from plats 160 and 170, Truckee-Carson Experiment Farm. Original nitrate present in samples from plat 160: Depth, 0 to 6 inches, 8.64 parts per million; 6 to 12 inches, 2.88; 12 to 18 inches, 4.8; 18 to 24 inches, 6; 24 to 36 inches, 4.8. From plat 170: Depth, 0 to 6 inches, 4.32 parts per million; 6 to 12 inches, 6; 12 to 18 inches, 3.84; 18 to 24 inches, 3.6; 24 to 36 inches, 3.

depth of 18 inches; below this it is very heavy, but below 26 and 30 inches it is again lighter in texture. At the time of sampling, this plat was supporting a fine growth of alfalfa. Plat 170 is in the northeast corner of the same field, and was very similar except that the

[1] This field had been irrigated a short time before the samples were collected.

surface was a little more compact and the alfalfa was practically a failure. The nitrification curves show the same general variations, but the one of the poor soil is consistently below that of the productive soil.

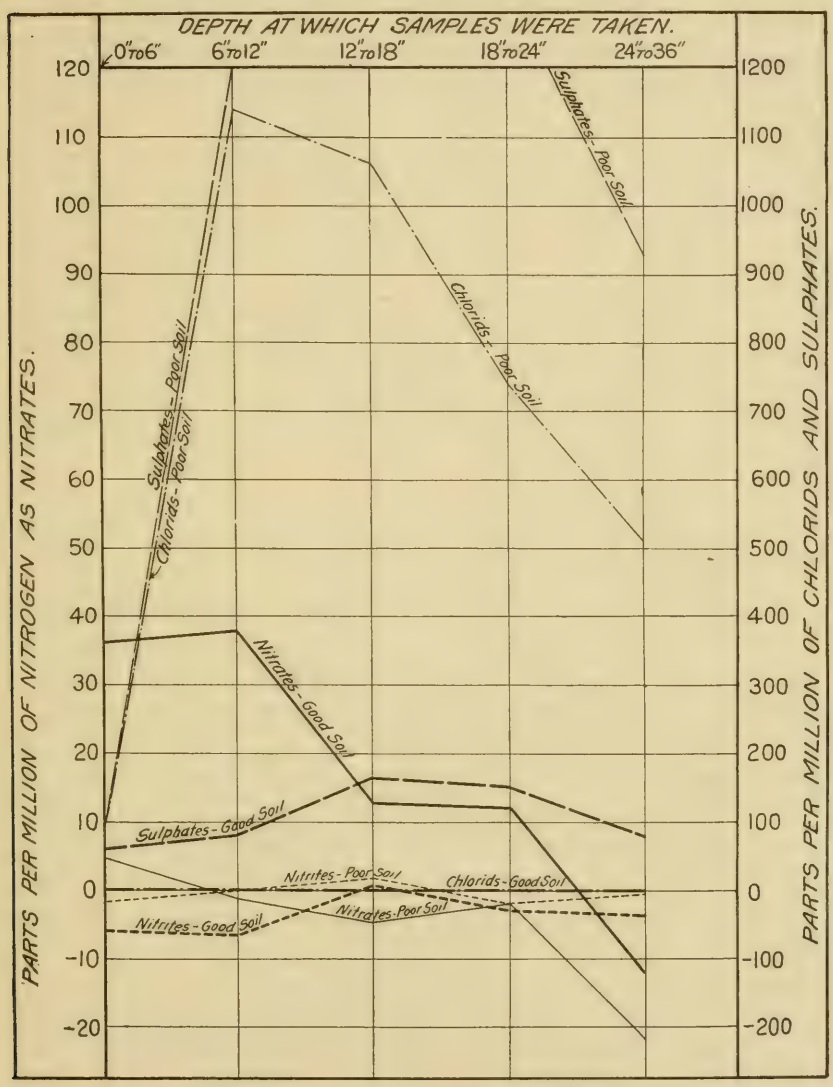

FIG. 6.—Diagram showing the nitrification of ammonium sulphate in samples of soil from different depths from plat 180 (poor soil) and plat 190 (good soil), Truckee-Carson Experiment Farm. Original nitrate present in samples from plat 180: Depth, 0 to 6 inches, 2 parts per million; 6 to 12 inches, 3.5; 12 to 18 inches, 8.25; 18 to 24 inches, 4.5; 24 to 36 inches, 25.75. From plat 190: Depth, 0 to 6 inches, 4.5 parts per million; 6 to 12 inches, 15.75; 12 to 18 inches, 11.25; 18 to 24 inches, 20.75; 24 to 36 inches, 21.75.

Plats 180 and 190 are located upon poor and good spots. The texture of the samples is very similar, both being sandy, but the surface of plat 180, the unproductive soil, is hard and compact as if

held together by some cementing material. As shown in figure 6, the nitrifying power of samples from plat 180 is almost nothing. In this figure the chlorid and sulphate curves are of interest, as those of plat 180, the poor soil, are far above those of plat 190, the good soil.[1]

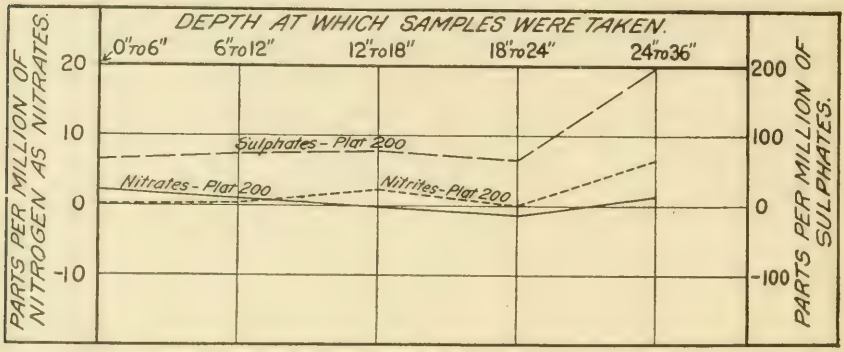

FIG. 7.—Diagram showing the nitrification of ammonium sulphate in samples of soil from different depths from plat 200, Truckee-Carson Experiment Farm. Original nitrate present in samples: Depth, 0 to 6 inches, 7.68 parts per million; 6 to 12 inches, 5.8; 12 to 18 inches, 3.93; 18 to 24 inches, 4.32; 24 to 36 inches, 1.82.

Figures 7 to 10, inclusive, show the nitrifying power of samples of soil from plats 200, 210, 220, and 230. They are in fields which have only recently been leveled and irrigated; in fact, 1909 was the first year they had been cropped. They produced a fair crop of barley, but the

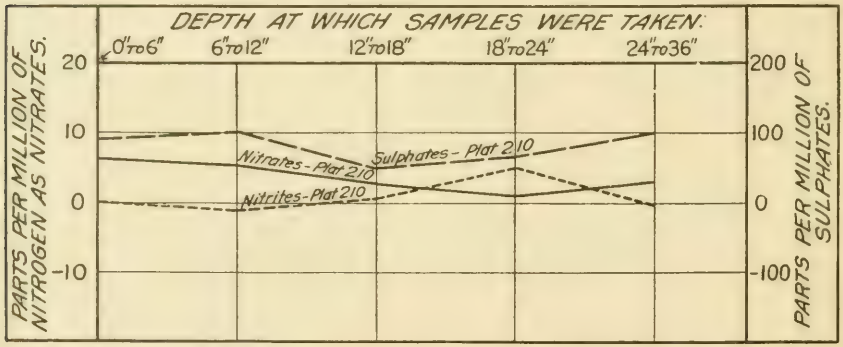

FIG. 8.—Diagram showing the nitrification of ammonium sulphate in samples of soil from different depths from plat 210, Truckee-Carson Experiment Farm. Original nitrate present in samples: Depth, 0 to 6 inches, 2.66 parts per million; 6 to 12 inches, 4.8; 12 to 18 inches, 4.16; 18 to 24 inches, 3; 24 to 36 inches, 2.

young alfalfa sown in the barley was doing only fairly well. The curves from all of these plats show a very low nitrifying power, yet a glance at the figures shows that nitrates were present in moderate quantities in the original samples.

[1] Bridge readings on these samples were made by Mr. Jensen.

Figures 11 and 12 present the results obtained from samples of soil from plats 240, 250, 260, and 270. The fields in which these plats are

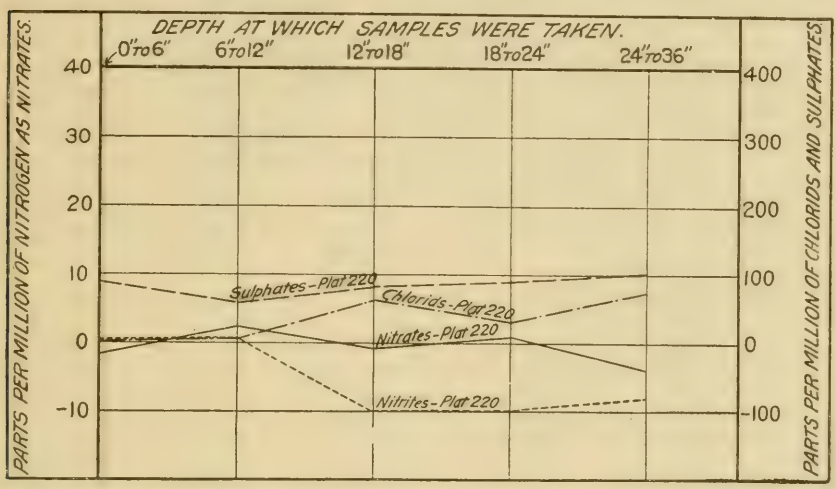

FIG. 9.—Diagram showing the nitrification of ammonium sulphate in samples of soil from different depths from plat 220, Truckee-Carson Experiment Farm. Original nitrate present in samples: Depth, 0 to 6 inches, 7.68 parts per million; 6 to 12 inches, 6.91; 12 to 18 inches, 10; 18 to 24 inches, 5.64; 24 to 36 inches, 6.

located have been merely leveled and left fallow, receiving regular applications of irrigation water. The field containing plats 240 and 250 is never cultivated, while that containing plats 260 and 270 is

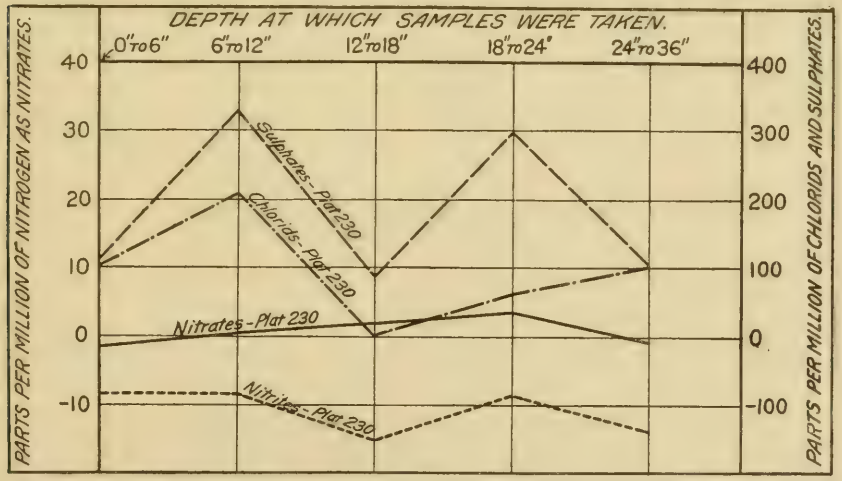

FIG. 10.—Diagram showing the nitrification of ammonium sulphate in samples of soil from different depths from plat 230, Truckee-Carson Experiment Farm. Original nitrate present in samples: Depth, 0 to 6 inches, 10 parts per million; 6 to 12 inches, 8.16; 12 to 18 inches, 5; 18 to 24 inches, 4.56; 24 to 36 inches, 9.6.

cultivated according to thorough summer-fallow methods. As the conditions are abnormal it is not surprising that the curves of chlorids

and sulphates, as well as the curve showing nitrification, should be so erratic and variable.

Figure 13 shows the nitrifying power of samples from plats 280 and

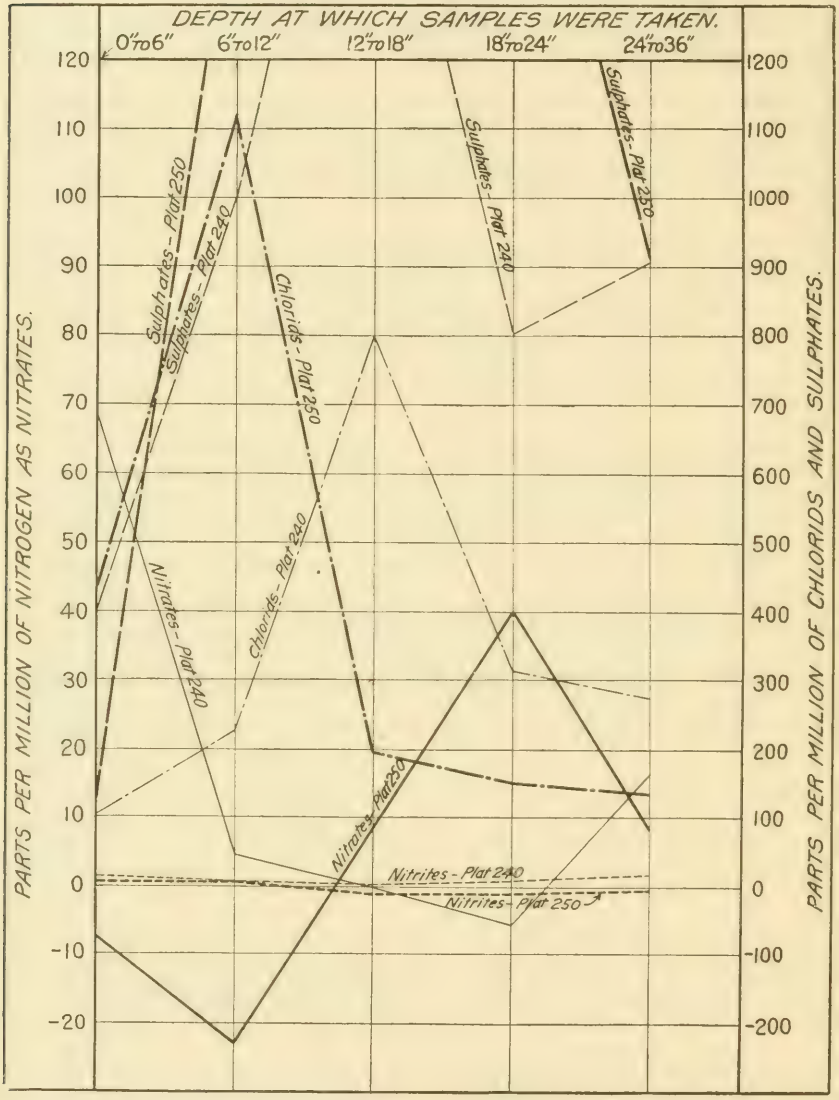

FIG. 11.—Diagram showing the nitrification of ammonium sulphate in samples of soil from different depths from plats 240 and 250, Truckee-Carson Experiment Farm. Original nitrate present in samples from plat 240: Depth, 0 to 6 inches, 6.8 parts per million; 6 to 12 inches, 8; 12 to 18 inches, 10.4; 18 to 24 inches, 6; 24 to 36 inches, 5. From plat 250: Depth, 0 to 6 inches, 28 parts per million; 6 to 12 inches, 48; 12 to 18 inches, 6; 18 to 24 inches, 5.2; 24 to 36 inches, 7.

290, located in an old alfalfa field just north of Fallon. These soils are very productive, and it was expected that they would show a greater nitrifying power than they did. This may possibly be

explained, however, by the original high nitrate content of the soil, as there is often a tendency for the nitrifying power of a soil to decrease as nitrates accumulate.

NITRIFICATION OF SAMPLES IN SOLUTION.

In order to further test for the presence of nitrifying bacteria and also to study some of their characteristics, inoculations were made

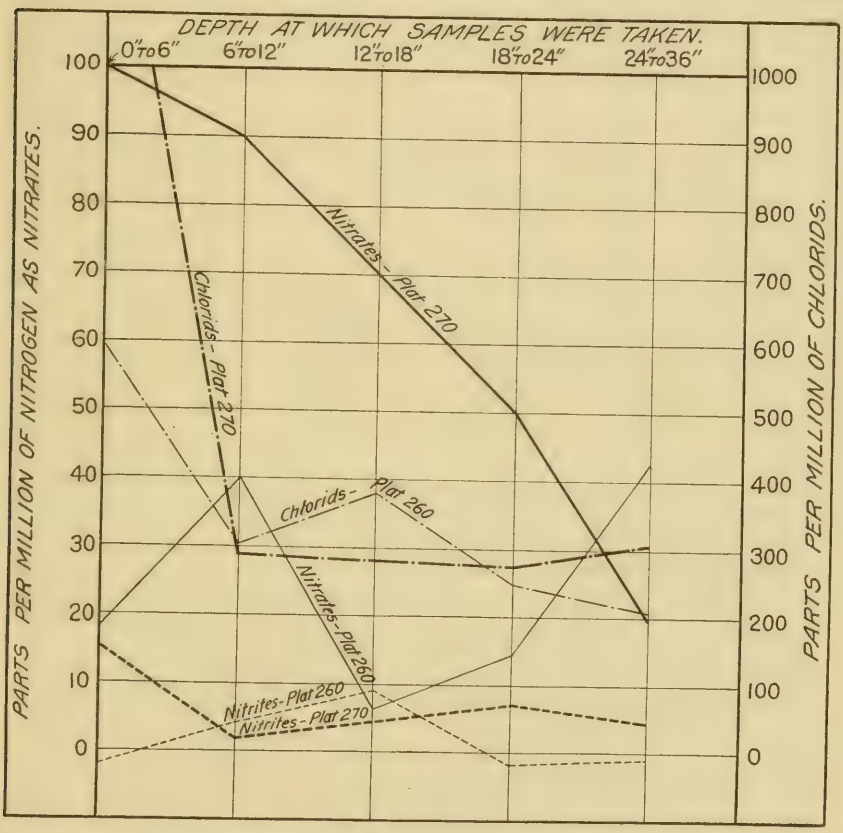

FIG. 12.—Diagram showing the nitrification of ammonium sulphate in samples of soil from different depths from plats 260 and 270, Truckee-Carson Experiment Farm. Original nitrate present in samples from plat 260: Depth, 0 to 6 inches, 62 parts per million; 6 to 12 inches, 30; 12 to 18 inches, 18.75; 18 to 24 inches, 35.7; 24 to 36 inches, 30. From plat 270: Depth, 0 to 6 inches, 100 parts per million; 6 to 12 inches, 27.7; 12 to 18 inches, 50; 18 to 24 inches, 40; 24 to 36 inches, 50.

into media consisting entirely of inorganic material which is not suitable for the growth of saprophytic bacteria.[1] Curves have not been plotted from the data thus obtained, as the conditions were too abnormal to warrant considering the differences from a quantitative

[1] Winogradsky and Omelianski's Fluid Culture-Medium for Isolating the Nitrate Bacteria from Soils, and Winogradsky and Omelianski's Fluid Culture-Medium for Isolating the Nitrite Bacteria from Soils. Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten, vol. 5, pt. 2, 1899, pp. 537–549.

standpoint. The results are all expressed in Table I as parts of nitrogen per million of the solution.

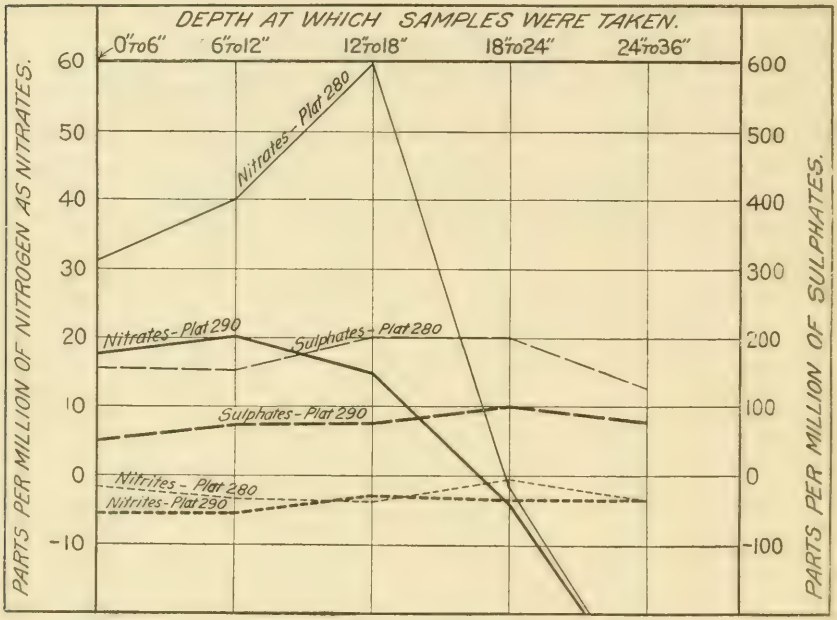

FIG. 13.—Diagram showing the nitrification of ammonium sulphate in samples of soil from different depths from plats 280 and 290, Truckee-Carson Experiment Farm. Original nitrate present in samples from plat 280: Depth, 0 to 6 inches, 12 parts per million; 6 to 12 inches, 10; 12 to 18 inches, 6; 18 to 24 inches, 15; 24 to 36 inches, 62.5. From plat 290: Depth, 0 to 6 inches, 60 parts per million; 6 to 12 inches, 60; 12 to 18 inches, 55.4; 18 to 24 inches, 60; 24 to 36 inches, 60.

TABLE I.—*Nitrification in solution of samples of soil from plats 100, 110, 180, 190, 220, 260, 270, and 280,*[1] *Truckee-Carson Experiment Farm. Incubated at 28° C.*

No. of plat.	Depth of soil.	Ammonia to nitrite (parts per million).[2]		Nitrite to nitrate (parts per million)[3]	
		6 days.	12 days.	10 days.	20 days.
	Inches.				
100	0–6	6.50	25	91.60	96.00
	6–12	5.00	25	64.80	60.00
	12–18	6.50	18	86.40	81.60
110	0–6	.50	15	.....	.....
	6–12	3.25	20	.....	.....
	12–18	8.00	25	.....	.....
180	0–6	0.00	15	24.00	86.40
	6–12	0.00	15	3.60	2.40
	12–18	0.00	00	2.40	3.00
190	0–6	1.00	16	72.00	74.00
220	0–6	0.00	00	13.20	80.00
	6–12	0.00	00	3.60	5.32
	12–18	0.00	00	2.40	5.60
260	0–6	5.00	17	76.80	74.00
	6–12	3.00	10	57.60	54.40
	12–18	3.00	10	2.88	17.55
270	0–6	6.50	20	9.60	43.20
	6–12	0.00	00	8.64	60.00
280[1]	0–6	5.75	20	21.60	81.60
	6–12	1.00	20	40.80	81.60
	12–18	1.00	20	38.40	81.60

[1] Plat 280 is located in an old alfalfa field one-fourth mile north of Fallon.
[2] Used medium for isolating nitrite bacteria. [3] Used medium for isolating nitrate bacteria.

It will be seen that the nitrifiers and especially the nitrate bacteria develop quite well in solutions. It should be noted that the only samples that failed to produce nitrites were those taken at 6-inch, 12-inch, and 18-inch depths from plat 220, which failed to nitrify in soil. (See fig. 9.) This soil, however, produced nitrates quite readily. This suggests the possibility that the lack of nitrification in this soil may be due to lack of nitrite bacteria.

CHLORIDS AND SULPHATES.

In alkali studies it is recognized that as a rule the chlorid type is more injurious to ordinary farm crops than the sulphate type. Further, in some investigations in the soils of the arid regions it has been found

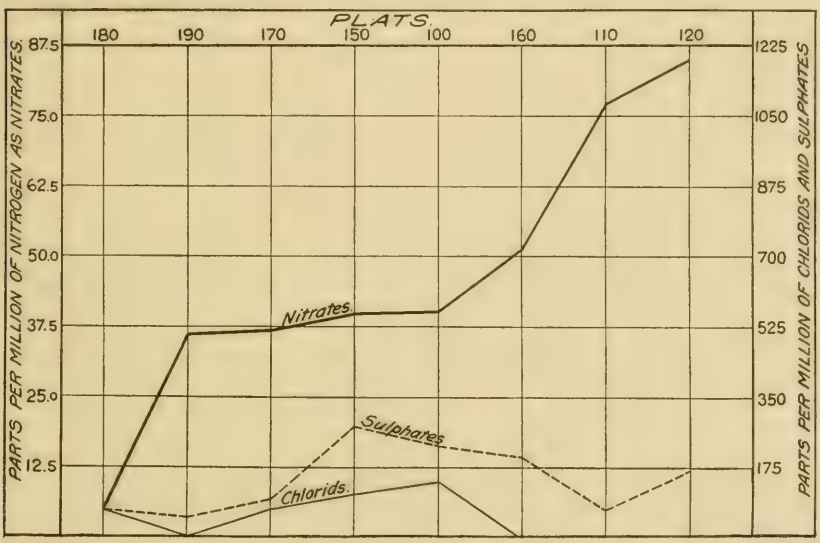

FIG. 14.—Diagram showing the relation between the quantity of alkali and the nitrification in samples of soil from plats 180, 190, 170, 150, 100, 160, 110, and 120, Truckee-Carson Experiment Farm. Samples taken from depths of 0 to 6 inches.

that high nitrates correlate with the sulphate type, while low nitrates are usually associated with the chlorid type. It was thought, therefore, that it would be of interest in connection with this work to study the relation of chlorids and sulphates to the nitrifying power.

In plotting these curves the different plats are arranged in such an order that the nitrification of ammonium sulphate by the different samples, which is the index of the difference of their powers of nitrification, forms an ascending series. Four diagrams are presented (figs. 14 to 17), one for each depth from which samples of soil were taken. Figure 14, representing the surface samples, shows no relation between the concentration of soluble salts and nitrifying power. Figures 16 and 17, representing the deeper samples, are

not in close agreement, although high alkali consisting of both chlorids and sulphates is apparently correlated with low nitrification. Little if any difference is to be noted between the effect of the chlorid and the sulphate types of alkali.

DENITRIFICATION.

In order to test for the presence of denitrifying bacteria several inoculations were made into Dunham's solution containing 0.2 per

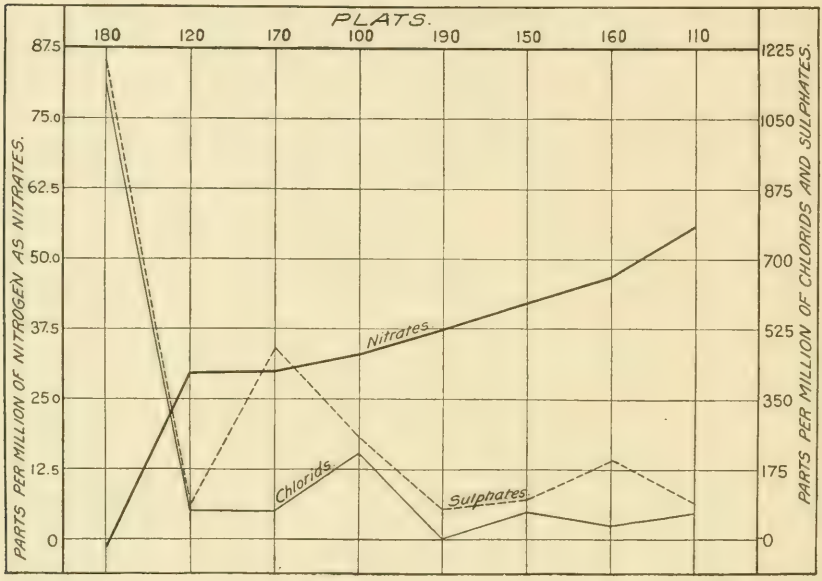

FIG. 15.—Diagram showing the relation between the quantity of alkali and the nitrification in samples of soil from plats 180, 120, 170, 100, 190, 150, 160, and 110, Truckee-Carson Experiment Farm. Samples taken from depths of 6 to 12 inches.

cent of potassium nitrate, and the free nitrogen gas evolved was measured. This medium favors the growth of this class of bacteria. The conditions thus produced are abnormal and the quantitative differences shown in Table II should not be taken too seriously. It will be seen from the table that denitrifying bacteria are present and active in almost all of the soils tested.

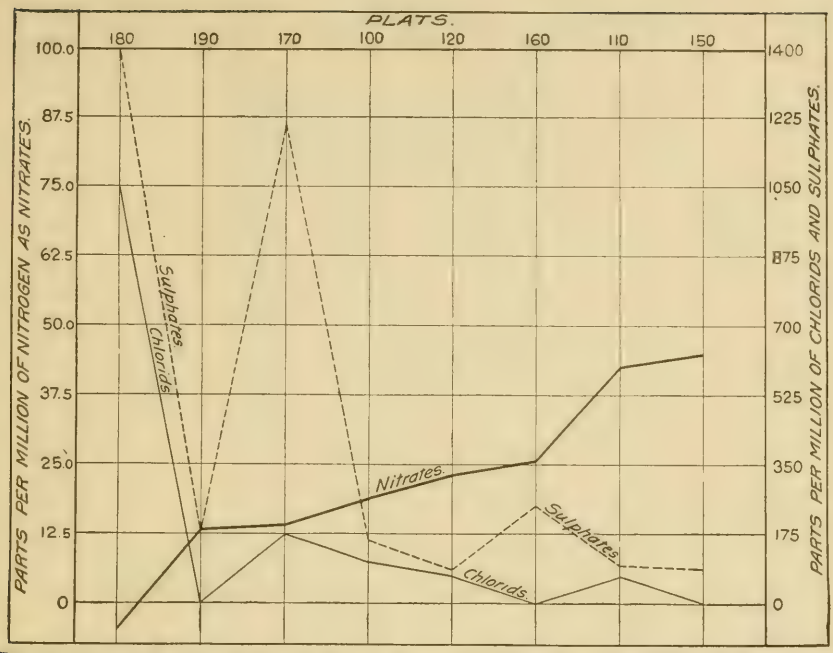

FIG. 16.—Diagram showing the relation between the quantity of alkali and the nitrification in samples of soil from plats 180, 190, 170, 100, 120, 160, 110, and 150, Truckee-Carson Experiment Farm. Samples taken from depths of 12 to 18 inches.

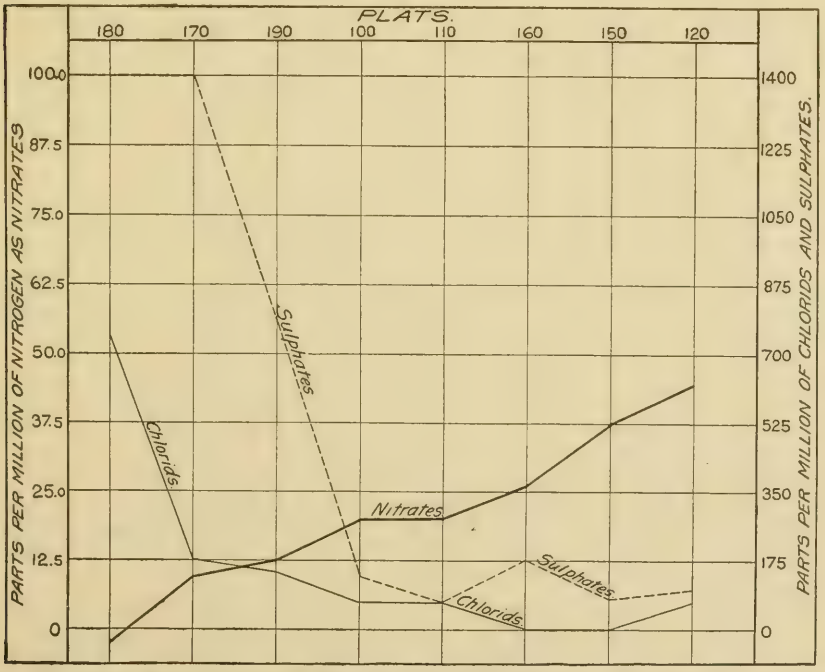

FIG. 17.—Diagram showing the relation between the quantity of alkali and the nitrification in samples of soil from plats 180, 170, 190, 100, 110, 160, 150, and 120, Truckee-Carson Experiment Farm. Samples taken from depths of 18 to 24 inches.

TABLE II. *Denitrification in solution of samples of soil from plats 180, 190, 230, 260, 270, 280,[1] and 290,[1] Truckee-Carson Experiment Farm.*

No. of plat.	Depth of soil.	Gas formed in 7 days.	Gas formed in 15 days.
	Inches.	*Per cent.*	*Per cent.*
180	0–6	25	25
	6–12	20	30
	12–18	20	25
190	0–6	22	32
	6–12	30	40
	12–18	32	42
230	0–6	20	30
	6–12	21	30
	12–18	20	35
260	0–6	40	53
	6–12	15	23
	12–18	20	30
270	0–6	20	30
	6–12	20	30
	12–18	20	30
280[1]	0–6	00	00
	6–12	Trace.	Trace.
	12–18	Trace.	Trace.
290[1]	0–6	22	40
	6–12	10	18
	12–18	45	62

[1] Plats 280 and 290 are located in an old alfalfa field one-fourth mile north of Fallon.

RELATIVE NUMBERS OF BACTERIA IN DIFFERENT SOILS.

An estimation of the number of bacteria in a gram of soil that would develop aerobically upon beef agar was made for many of the sampling plats in accordance with the method previously described, the results of which are shown in Table III. In accord with the reports of other investigators, [1] the data presented in Table III clearly show that the numbers of bacteria found in the different samples bear no consistent relation to the fertility or crop-producing power of the respective fields.

No attempt was made to determine the relative numbers of protozoa in samples of soil from the good and poor areas. If the development of protozoa is determined by their food supply,[2] in other words, by the numbers of bacteria existing in the soil, it is obvious that in this region the crop-producing power can not be limited [3] by the abundance of protozoa.

[1] Löhnis, F. Ein Beitrag zur Methodik der bakteriologischen Bodenuntersuchung. Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten, pt. 2, vol. 12, no. 6–8, June 24, 1904, pp. 262–267.

Chester, Frederick D. The Bacteriological Analysis of Soils. Bulletin 65, Delaware College Agricultural Experiment Station, March 1, 1904.

Voorhees, Edward B., and Lipman, Jacob G. A Review of Investigations in Soil Bacteriology. Bulletin 194, Office of Experiment Stations, U. S. Dept. of Agriculture, October 26, 1907.

[2] Russell, E. J., and Hutchinson, H. B. The Effect of Partial Sterilization of Soil on the Production of Plant Food. Contributions from the Laboratory of the Rothamsted Experimental Station, October, 1909, pp. 111–144.

[3] Hall, A. D. The Fertility of the Soil. Science, n. s., vol. 32, no. 820, September 16, 1910, pp. 363–371.

TABLE III.—*Number of bacteria per gram of soil and nitrifying power of samples from plats 10, 20, 30, 40, 180, 190, 290,[1] 240, 260, and 270, Truckee-Carson Experiment Farm.*

No. of plat.	Depth of soil.	Number of bacteria per gram.	Nitrifying power of soils (parts per million).	Character of soil.
	Inches.			
10	0–6	435,000	4.4	Very poor.
	6–12	251,000	2.0	
	12–18	26,650	.0	
	18–24	146,250	3.0	
	24–36	1,000	.0	
20	0–6	19,500	54.2	Very productive.
	6–12	11,250	6.8	Good growth of alfalfa.
	12–18	30,000	1.0	
	18–24	4,500	.0	
	24–36	3,000	.0	
30	0–6	160,000	3.0	Poor and compact.
	6–12	65,000	.0	
	12–18	262,000	.0	
	18–24	19,855	.0	
	24–36	10,000	.0	
40	0–6	210,000	20.4	Productive.
	6–12	20,000	4.0	Good growth of alfalfa.
	12–18	135,000	1.0	
	18–24	45,000	.0	
	24–36	1,000	.0	
180	0–6	60,000	4.72	Very poor.
	6–12	175,000	1.54	Alkali high. (See fig. 6.)
	12–18	180,000	4.32	
	18–24	4,000	2.54	
190	0–6	3,600	36.30	Productive.
	6–12	168,000	37.45	
	12–18	1,554,000	12.75	
	18–24	704,000	12.25	
290[1]	0–6	273,000	30.00	Productive.
	6–12	396,000	20.00	Old alfalfa field. (See fig. 13.)
	12–18	262,500	14.60	
	18–24	327,000	4.00	
240	0–6	52,000	69.00	Fallow. (See fig. 11.)
	6–12	78,700	4.50	
	12–18	56,000	.40	
260	0–6	81,000	18.00	Fallow. (See fig. 12.)
	6–12	153,300	40.00	
270	0–6	72,000	100.00	
	6–12	2,790,000	92.30	

[1] Plat 290 is located in an old alfalfa field one-fourth mile north of Fallon.

DETAILED STUDY OF SOIL TYPICAL OF EXTENSIVE AREAS.

Plats 300 to 350 are representative of a somewhat extensive type of soil of the Truckee-Carson project. This soil is very unproductive as a rule, almost barren in many cases, yet all through it, wherever properly leveled and irrigated, are spots of a few square rods in area that are normal and productive. The difference between these two conditions seemingly can not be explained by any of the now known causes of infertility. There is a certain difference in texture, or rather in the physical properties; the productive soil is loose and sandy, while the unproductive type, although sandy, contains a small quantity of clay which when shaken up with water remains suspended indefinitely and the soil cements on drying. These physical differences, while no doubt factors, do not seem adequate causes of the extremely low fertility. The total alkali content is not high enough

to produce toxic effects, and a lack of mineral plant food in the virgin soils is almost out of the question.

Both soils are low in organic matter, as are all arid soils. Good soil management in other somewhat similar regions would indicate that the addition of organic matter to these soils in the form of barnyard manure or green manure should produce beneficial physicochemical effects, and such treatments have been applied somewhat extensively as a matter of experiment during the last two or three years. The poor soil apparently has not been benefited to a noticeable degree. The good soil has been somewhat improved, although even here the improvement has not been striking. A minute field examination of these good and poor spots a year or more after they had received applications of organic matter revealed a remarkable difference; all traces of the organic material had disappeared from the fertile spots, while the larger part of the manure added to the infertile spots was in an almost perfect state of preservation. Another peculiar difference was that in the poor spots, at depths of 6 to 28 inches, an irregularly distributed, dark-colored, foul-smelling layer was found, undoubtedly due to the presence of a peculiar organic decomposition product, while such a layer was never found associated with good soil. It should not be inferred from this description that this black layer was found only where organic matter has been added as a treatment; it was quite generally distributed through these infertile soils and is presumably due to the decay of such material as was turned into the soil when it was first reclaimed, such as sagebrush, greasewood, rabbit brush, and other desert plants, together with the roots of these plants which have been accumulating for long periods of time. Laboratory samples showed that this black substance was easily oxidized, for when a sample was taken to the laboratory, dried, and subsequently moistened for physiological experiments, all traces of the black color and peculiar odor disappeared.

These unusual conditions of the decay of organic matter are necessarily somewhat closely associated with improper bacteriological conditions; that is, the improper utilization of organic fertilizers is due either to an improperly balanced or incomplete bacterial flora or to physical or chemical conditions preventing the performance of the normal activities of the bacteria present.

Titrations of some of the aqueous extracts indicated that sodium carbonate (black alkali) was present in the poor soils but not in the good soils. It was also apparent that calcium sulphate and gypsum, when applied in large quantities, produced a decided effect in flocculating the finely divided or colloidal clays. Samples were collected with a sterile spatula from the sides of freshly dug holes and placed in sterile containers. Portions of these samples were inoculated into

Winogradsky's solutions and also into flasks of sterile water, from which counts were made. The remaining portions of the samples were then emptied on clean sheets of paper in the culture room and left to dry under conditions as free as possible from chance contaminations. Fifty-gram portions from each sample were removed for original nitrate determinations, and another equal portion was replaced in the original containers, brought up to optimum moisture content with 5 cubic centimeters of 0.4 per cent sulphate of ammonia and distilled water, incubated for two weeks at 28° C., and the nitrification determined. A duplicate series to which was added a 2 per cent solution of calcium sulphate was prepared. At the beginning of the incubation period the total weight of the container and soil at optimum moisture was taken, and the loss from evaporation was restored with sterile distilled water at 3-day intervals during the incubation period. The results of the experiment appear in Tables IV and V.

TABLE IV.—*Effect of calcium sulphate upon nitrification in samples of soil from plats 300 and 310, Truckee-Carson Experiment Farm, representing poor soil conditions. Incubated 15 days at 28° C.*

NO CALCIUM SULPHATE ADDED TO SAMPLES.

No. of plat.	Depth of soil.	Nitrogen as nitrite (parts per million).			Nitrogen as nitrate (parts per million).		
		Original.	Final.	Gain.	Original.	Final.	Gain.
	Inches.						
300	0–6	1.2	5.60	4.40	9.12	56.40	46.28
	6–12	2.0	3.12	1.12	7.68	00.00	— 7.68
	12–18	1.2	1.68	.48	6.14	00.00	— 6.14
	18–24	.0	1.20	1.20	4.56	00.00	— 4.56
310	0–6	1.0	7.28	6.28	4.80	96.00	91.20
	6–12	1.0	2.10	1.10	1.60	00.00	— 1.60
	12–18	1.0	.80	— .20	4.32	00.00	— 4.32
	18–24	1.0	1.20	.20	3.07	00.00	— 3.07

WITH 2 PER CENT CALCIUM SULPHATE ADDED TO ALL SAMPLES.

300	0–6	1.2	2.80	1.60	9.12	57.00	47.88
	6–12	2.0	2.80	.80	7.68	00.00	— 7.68
	12–18	1.2	2.70	1.50	6.14	00.00	— 6.14
	18–24	.0	.88	.88	4.56	00.00	— 4.56
310	0–6	1.0	4.00	3.00	4.80	96.00	91.20
	6–12	1.0	2.40	1.40	1.60	1.20	— .40
	12–18	1.0	1.68	.68	4.32	0.00	4.32
	18–24	1.0	1.56	.56	3.07	0.00	— 3.07

TABLE V.—*Effect of calcium sulphate upon nitrification in samples of soil from plat 320, Truckee-Carson Experiment Farm, representing good soil conditions. Incubated 15 days at 28° C.*

NO CALCIUM SULPHATE ADDED TO SAMPLES.

No. of plat.	Depth of soil.	Nitrogen as nitrite (parts per million).			Nitrogen as nitrate (parts per million).		
		Original.	Final.	Gain.	Original.	Final.	Gain.
	Inches.						
320	0–6	1.4	4.00	2.60	3.84	80.64	76.80
	6–12	1.5	1.20	−.30	18.24	76.80	58.56
	12–18	.0	1.60	1.60	15.90	5.00	−10.90
	18–24	.8	1.40	.60	15.36	0.00	−15.36

WITH 2 PER CENT CALCIUM SULPHATE ADDED TO ALL SAMPLES.

320	0–6	1.4	5.00	3.60	3.84	81.60	77.76
	6–12	1.5	1.68	.13	18.24	76.80	58.56
	12–18	.0	1.82	1.82	15.90	4.56	−11.36
	18–24	.8	.60	−.20	15.36	.00	−15.36

The gain in nitrates, or the nitrifying power of these samples of soil, is shown in figure 18.

These experiments on nitrification indicate that the difference in productiveness is not due to a suspension of nitrification, and also that it is not due to the presence of sodium carbonate, as the addition of calcium sulphate to the samples had absolutely no effect: the treated and untreated samples could really be considered duplicates. It would seem also that the infertility or the lack of decay of organic substances is not due to lack of air. It might be argued that laboratory conditions were not such as would favor the compacting or cementing of the samples, yet it must be remembered that the cornfield containing plats 300, 310, and 320 was kept well cultivated and no crust was allowed to form during the growing season. Tables VI and VII show the nitrification of the different samples in Winogradsky and Omelianski's media.

TABLE VI.—*Nitrite formed from ammonia by samples of soil from plats 330, 340, and 350, Truckee-Carson Experiment Farm, in medium for nitrite bacteria. Incubated at 28° C.*

No. of plat.	Depth of of soil.	Nitrite formed in 5 days (parts per million).	Nitrite formed in 10 days (parts per million).	Character of soil.
	Inches.			
330	0–6	0.0	4.8	Poor.
	6–12	.0	.0	
	12–18	.0	.0	
	18–24	.0	.0	
340	0–6	9.6	10.4	Do.
	6–12	12.8	12.8	
	12–18	12.8	12.8	
	18–24	.0	12.2	
350	0–6	.0	Trace.	Good.
	6–12	.0	4.8	
	12–18	.0	.0	
	18–24	.0	.0	

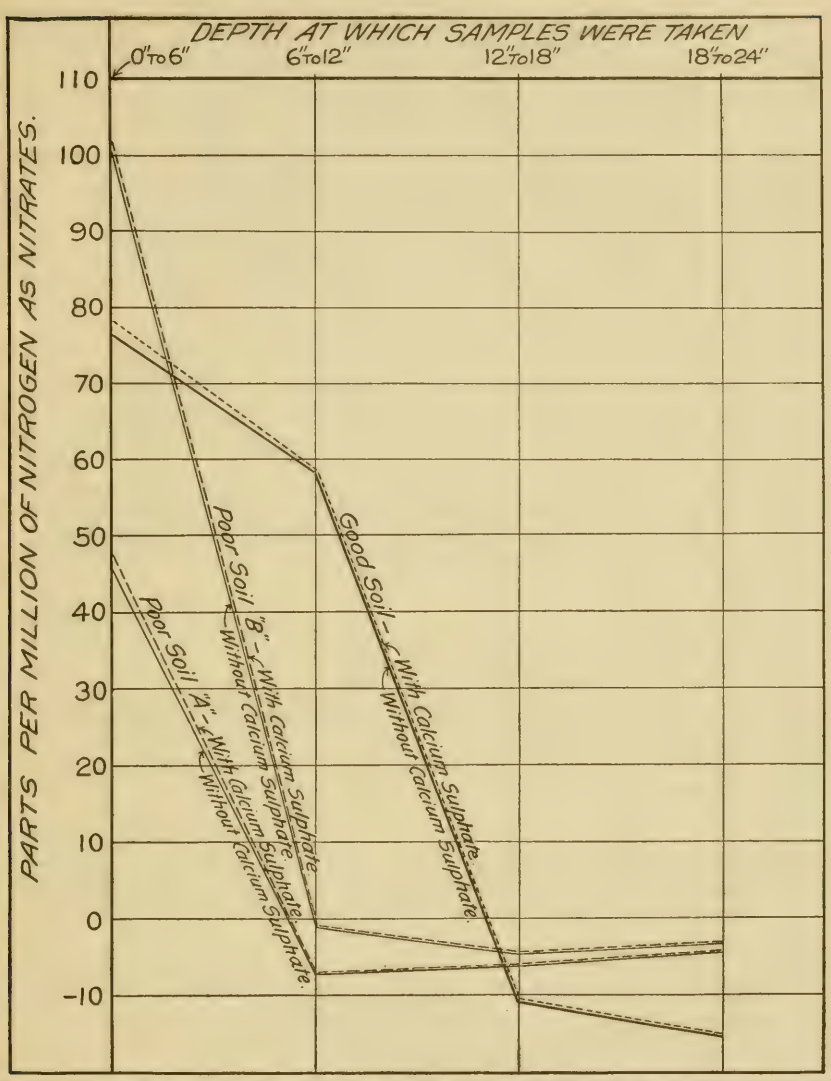

FIG. 18.—Diagram showing the effect of calcium sulphate upon nitrification of ammonium sulphate in samples of soil from plat 300, Truckee-Carson Experiment Farm, representing poor soil "*A*"; plat 310, representing poor soil "*B*"; and plat 320, representing good soil.

TABLE VII.—*Nitrate formed from nitrite by samples of soil from plats 330 and 350, Truckee-Carson Irrigation Project, in medium for nitrate bacteria. Incubated at 28° C.*

No. of plat.	Depth of soil.	Nitrate formed in 10 days (parts per million).	Nitrate formed in 20 days (parts per million).	Character of soil.
	Inches.			
330	0–6	24.00	90.32	Poor.
	6–12	19.68	90.32	
	12–18	12.60	81.28	
	18–24	19.80	90.32	
350	0–6	12.50	54.19	Good.
	6–12	24.00	72.25	
	12–18	.....	.....	
	18–24	4.12	90.32	

The fact that nitrification was feeble in the good soil and also in one of the poor soils should not be overemphasized, for soils that will nitrify under normal conditions frequently fail to do so in solutions. On the other hand, the rapidity with which the nitrate bacteria worked in solutions, even when they failed to do so in the soil, is interesting and almost without parallel. It is not surprising that a soil should fail to nitrify in solution, but it is remarkable that samples which failed to nitrify when kept warm and moist—ideal conditions for nitrification—should produce nitrates rapidly when inoculated into solutions.

The production of ammonia from organic material by soil bacteria furnishes a means of measuring the power of the soil flora to break down nitrogenous organic substances. Thus it would seem that the soils of the plats in which organic matter remained indefinitely in a

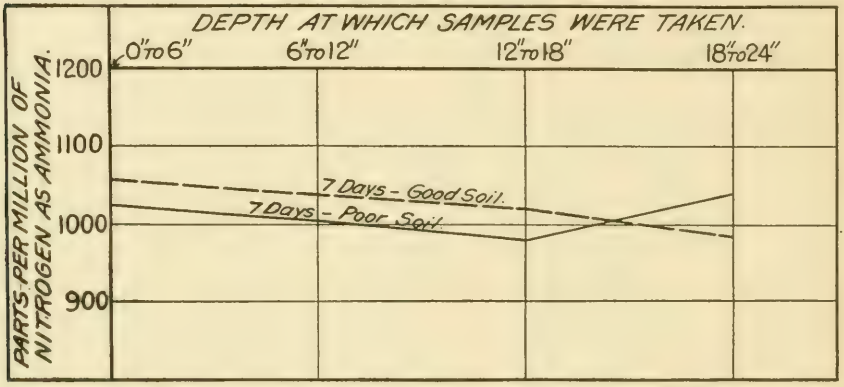

FIG. 19.—Diagram showing the ammonification of peptone in 7 days in samples of soil from plat 350 (good soil) and from plats 330 and 340 (poor soil), Truckee-Carson Experiment Farm.

state of preservation must have a very low ammonifying power. The medium described previously, consisting of 1.5 per cent peptone and inorganic salts, was inoculated with samples from plats 330, 340, and 350, and the ammonia produced determined at 10-day and 20-day incubation periods by distillation with magnesia.[1] The results of this experiment are shown in figures 19 and 20.

As the ammonification of the samples of poor soil, plats 330 and 340, was very similar, the results are averaged and shown as a single curve.

The fact that there is no increase between the 7-day and 15-day periods indicates that the maximum had been reached before any

[1] Dr. J. G. Lipman has recently suggested the use of dried blood as a source of nitrogen for work of this character. See Lipman, Jacob G., and Brown, Percy E., "Experiments on Ammonia and Nitrate Formation in Soils," in Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten, pt. 2, vol. 26, no. 20–24, April 9,1910, pp. 590–632.

determinations were made. Yet these results show conclusively that in both good and poor soils there are large numbers of ammonifiers which are physiologically active if proper conditions are provided for them to develop. The relative differences in their ammonifying power and whether or not there are conditions in the soil to prevent their normal activities remain to be shown by further experiment.

Denitrification is of two kinds: The reduction of nitrates to lower forms or transformation into organic form, and the complete breaking down of the nitrogenous substance with the evolution of free nitrogen as a gas. Either of these processes could be a source of infertility.

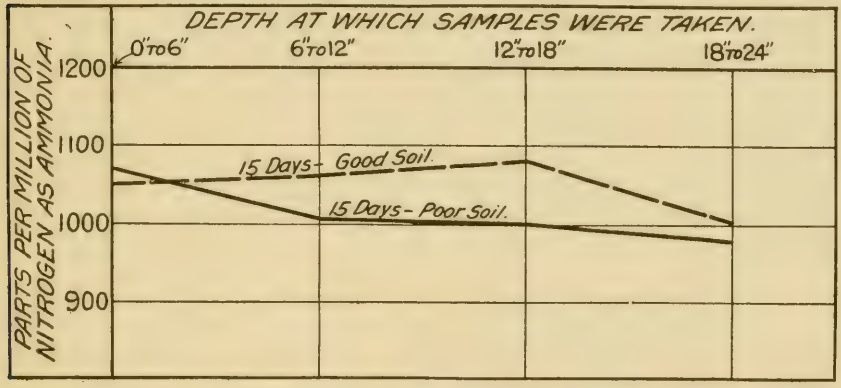

FIG. 20.—Diagram showing the ammonification of peptone in 15 days in samples of soil from plat 350 (good soil) and from plats 330 and 340 (poor soil), Truckee-Carson Experiment Farm.

The evolution of free nitrogen was determined by measuring the nitrogen gas produced from peptone-nitrate solutions at intervals of 7 and 15 days. The results are rather erratic, as is shown in Table VIII.

TABLE VIII.—*Denitrification by samples of soil from plats 330, 340, and 350, Truckee-Carson Experiment Farm.*

No. of plat.	Denitrification. Depth of soil.	Gas formed in— 7 days.	Gas formed in— 15 days.	Character of soil.
	Inches.	*Per cent.*	*Per cent.*	
330	0–6	30	35	Poor.
	6–12	1	10	
	12–18	2	5	
	18–24	2	5	
340	0–6	35	40	Do.
	6–12	40	40	
	12–18	20	25	
	18–24	30	40	
350	0–6	2	7	Good.
	6–12	1	3	
	12–18	1	5	
	18–24	20	20	

Table IX shows the difference between the good and poor soils in regard to total numbers and distribution of bacteria. The difference in the floras is more strikingly brought out when we consider the difference in the colonies from the different soils. The plates from the 6-inch and 12-inch layers of plats 300 and 310, which show low numbers, chiefly contained peculiar colonies surrounded by a wine-colored diffusible pigment. The colony itself was but slightly colored and, surrounded as it was by this pigment, produced a very striking appearance on the plates. One plate from plat 310 was apparently a pure culture of this organism. Such a plate obtained from soil where the growth or flora is almost always rich and varied is very rare, and is the only unusual condition thus far encountered that seems to correlate consistently with the unusual conditions of infertility. This peculiar colony was never seen on soils from the fertile spots, and the fact that it was so predominately present in the infertile soils and in those strata in which the peculiar black layer occurred certainly indicates that further study should be made of this point. Microscopic examination of the colony showed that it was a micrococcus associated with a mold.

TABLE IX.—*Total number of bacteria present in 1-gram samples of soil from plats 300, 310, 320, 330, 340, and 350, Truckee-Carson Experiment Farm.*

No. of plat.	Depth of soil.	Number of bacteria per gram.	Character of soil.
	Inches.		
300	0–6	458,400	Poor.
	6–12	45,000	
	12–18	48,900	
	18–24	178,500	
310	0–6	1,930,500	Do.
	6–12	729,000	
	12–18	15,900	
	18–24	409,500	
320	0–6	507,000	Good.
	6–12	351,000	
	12–18	419,000	
	18–24	429,000	
330	0–6	1,335,000	Poor.
	6–12	915,000	
	12–18	840,000	
	18–24	1,197,000	
340	0–6	4,200,000	Do.
	6–12	525,000	
	12–18	4,620,000	
	18–24	3,780,000	
350	0–6	672,000	Good.
	6–12	.....	
	12–18	636,000	
	18–24	210,000	

CONCLUSIONS.

(1) Nitrifying, denitrifying, and ammonifying bacteria are well distributed and universally present in the soils of the Truckee-Carson Irrigation Project and become physiologically active if favorable conditions are provided for their development.

(2) The lack of proper decay and humification of organic matter in many of the unproductive soils is due either to unfavorable bacterial conditions brought about by certain physical and chemical conditions or to an unusual bacterial flora.

(3) The nitrifying bacteria in the soils of Fallon, Nev., are active at greater depths than in eastern soils and also seem to be unusually virile in solutions, although the data on these points are not conclusive.

(4) In general, the conditions at Fallon, as in any arid region, favor nitrification, which frequently becomes intense; the conditions rarely favor denitrification. Lack of nitrification, therefore, will not be a limiting factor in crop production, nor is there evidence of overnitrification or injury from excessive quantities of nitrate. Humification studies are probably of paramount importance.

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